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Experiments on Nitrogen Fixation in Cow's Rumen

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During the last few decades it has been shown that many bacteria of different types can fix gaseous nitrogen and use it as their nitrogen source. In ruminants the bacterial flora of the rumen is a complicated mixture of different bacteria. Therefore it is not surprising that in the rumen of the cow and the sheep it has been possible to find bacterial strains which in a proper nutrient substrate without combined nitrogen fix molecular nitrogen (Tóth). This does not, however, give evidence that nitrogen fixation occurs in the rumen, because the circumstances that are prevalent in the rumen are not suitable for such a process.

As during this decade it has been possible in this laboratory to demonstrate such an effective protein synthesis from urea and ammonia in the cow's rumen that it has been sufficient for an annual production of about 4000 kg milk, it was interesting to investigate whether also the utilization of molecular nitrogen takes place to a measurable extent in the rumen.

Methods

When studying whether molecular nitrogen is fixed in cow's rumen two kinds of experiments were performed with a cow which had a fistula.

In the first series of experiments about 20 ml rumen fluid was put immediately into a dialysis tube (diameter 15 mm), one end of the tube being closed. In addition about 20 ml nitrogen gas, containing 97 atom per cent ^{15}N, was passed into the tube. The other end of the tube was closed, too, and the tube was placed in a cloth bag in order to prevent breaking of the dialysis membrane. A long thread was attached to the bag. Several bags prepared in this way were pushed into the rumen through the fistula, which was then closed so that the ends of the threads were left outside the fistula. The bags were allowed to remain in the rumen for various times up to two hours, after which they were located by following the threads attached to them. When the bags had been removed from the rumen, bacterial action was stopped immediately either by adding ethanol or cooling the samples to -70°C. Such experiments were performed three times, the feeding of the cow being somewhat different each time.

In an experiment of the second type $^{15}N_2$ was mixed directly with the whole content of the rumen. A soft rubber stopper was pushed tightly into the fistula in the cow's flank. Through a hole in this stopper a 10 mm thick stainless steel tube was passed.

To the lower end of the tube inside the rumen a nylon ball, the diameter of which was 50 mm, had been attached. In this ball several tens of 1 mm holes had been drilled radially. The $^{15}N_2$ gas (97 atom per cent ^{15}N) was pumped into the rumen via this tube. While the gas was being passed into the rumen the tube was turned slowly so that the ball at its end moved through every part of the lower rumen, distributing the gas in very small bubbles to the whole content of the rumen. 950 ml gas was passed in during a period of 30 minutes. Again, several samples were taken during a period of two hours and they were treated in the same way as in the previous experiments.

In connection with all experiments, immediately before the particular test, control samples were taken from the rumen content in order to determine the nitrogen isotope ratio.

With all samples several isotope analyses were made, for example on total nitrogen, protein nitrogen, non-protein nitrogen etc. Also samples of faeces and urine were taken and analyzed.

Results

The occurrence of nitrogen fixation could not be demonstrated in the rumen.

With the measuring method used it was possible to detect such small differences in the isotope ratio as were to be expected in connection with an experiment of this kind. It is true that very small changes in the isotope ratio take place in general in chemical and physical processes, so that the interpretation of such small differences is difficult.

In the experiment in which nitrogen was passed directly into the rumen, the amount of nitrogen used can be regarded as small considering the large volume of the rumen. Vigorous formation of gas in the rumen sweeps out rapidly the nitrogen added, so that the $^{15}N_2$ concentration in the rumen during the experiment was probably small. The high price of ^{15}N, however, prevents the performing of a more thorough investigation.

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Reference

1. Tóth, L. Experientia IV (1948) 395.